(ii) *Reporting requirements.* (A) The fish early life stage toxicity test shall be completed and the final report submitted to the Agency within 2 years of the effective date of the final rule.

(B) Quarterly progress reports shall be submitted to the Agency beginning 90 days after the effective date of the final test rule.

(7) *Bioconcentration in fish*—(i) *Required testing.* A bioconcentration test shall be conducted with TBBPA using *Pimephales promelas* (fathead minnow) in accordance with § 797.1520 of this chapter.

(ii) *Reporting requirements.* (A) The bioconcentration test in fish shall be completed and the final report submitted to the Agency within 1 year of the effective date of the final rule.

(B) Quarterly progress reports shall be submitted to the Agency beginning 90 days after the effective date of the final test rule.

(8) *Bioconcentration in oyster*—(i) *Required testing.* A bioconcentration test shall be conducted with TBBPA using *Crassostrea virginica* (oyster) in accordance with § 797.1830 of this chapter.

(ii) *Reporting requirements.* (A) The bioconcentration test in oyster shall be completed and the final report submitted to the Agnecy within one year of the effective date of the final rule.

(B) Quarterly progress reports shall be submitted to the Agency beginning 90 days after the effective date of the final test rule.

(Information collection requirements have been approved by the Office of Management and Budget under Control Number 2070-0033)

[FR Doc. 86-10705 Filed 5-14-86; 8:45 am]
BILLING CODE 6560-50-M

---

40 CFR Part 799

[OPTS-42080; FRL-3001-9]

Triethylene Glycol Monomethyl, Monethyl, and Monobutyl Ethers; Proposed Test Rule

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is proposing that manufacturers and processors of triethylene glycol monomethyl ether (CAS No. 112-35-6), triethylene glycol monomethyl ether (CAS No. 112-50-5), and triethylene glyclo monobutyl ether (CAS No. 143-22-6) be required, under section 4 of the Toxic Substances Control Act (TSCA), to perform testing for these three chemicals for subchronic toxicity, developmental toxicity, neurotoxicity and developmental neurotoxicity, mutagenicity, reproductive toxicity, and oncogenicity. This is a two-stage rule. The subchronic toxicity, developmental toxicity and developmental neurotoxicity, the neurotoxicity and the lower-tier mutagenicity are in the first stage. Following the receipt of the first-stage data, EPA will review it and decide what further testing needs to be done in stage two. This proposed rule responds to the Interagency Testing Committee's (ITC's) designation of these three compounds for priority consideration for health effects testing.

DATES: Submit written comments on or before July 14, 1986. If persons request an opportunity to submit oral comment by June 30, 1986, EPA will hold a public meeting on this rule in Washington, DC. For further information on arranging to speak at the meeting see Unit VIII of this preamble.

ADDRESS: Submit written comments, identified by the document control number (OPTS-42080), in triplicate to: TSCA Public Information Office (TS-793), Office of Pesticides and Toxic Substances, Environmental Protection Agency, Rm. E-108, 401 M St., SW., Washington, DC 20460.

A public version of the administrative record supporting this action (with any confidential business information deleted) is available for inspection at the above address from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Wasington, DC 20460, Toll free: (800-424-9065); In Washington, DC: (554-1404); Outside the USA: (Operator—202-554-1404).

SUPPLEMENTARY INFORMATION: EPA is issuing a proposed test rule under section 4(a) of TSCA in response to the ITC's designation of triethylene glycol monomethyl, monoethyl, and monobutyl ether for health effects testing consideration.

I. Introduction

A. ITC Recommendation

TSCA (Pub. L. 94-469, 90 Stat. 2003 *et seq.*; 15 U.S.C. 2601 *et seq.*) established ITC under section 4(e) to recommend to EPA a list of chemicals to be considered for testing under section 4(a) of the Act.

ITC designated the three triethylene glycol ethers for priority testing consideration in its Sixteenth Report, published in the **Federal Register** of May 21, 1985 (50 FR 20930). ITC recommended pharmacokinetic and metabolic studies. Dependent upon the results of the pharmacokinetic and metabolic work, subchronic studies with emphasis on hematologic effects, as well as reproductive and developmental toxicity studies, should be performed. This document responds to ITC's designation.

B. Test Rule Development Under TSCA

Under section 4 (a) of TSCA, EPA shall by rule require testing of a chemical substance or mixture to develop appropriate test data if it finds that:

(A) (i) the manufacture, distribution in commerce, processing, use, or disposal of a chemical substance or mixture, or that any combination of such activities, may present an unreasonable risk of injury to health or the environment,

(ii) there are insufficient data and experience upon which the effects of such manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(iii) testing of such substance or mixture with respect to such effects is necessary to develop such data; or

(B) (i) a chemical substance or mixture is or will be produced in substantial quantities, and (I) it enters or may reasonably be anticipated to enter the environment in substantial quantities or (II) there is or may be significant or substantial human exposure to such substance or mixture,

(ii) there are insufficient data and experience upon which the effects of the manufacture, distribution in commerce, processing, use, or disposal of such substance or mixture or of any combination of such activities on health or the environment can reasonably be determined or predicted, and

(iii) testing of such substance or mixture with respect to such effects is necessary to develop such data.

In making section 4(a)(1)(A) findings, EPA considers both exposure and toxicity information to make the finding that the chemical may present an unreasonable risk. For the second finding under section 4(a)(1)(A), EPA examines toxicity studies to determine whether existing information is adequate to reasonably determine or predict the effects of human exposure to the chemical. In making the third finding that testing is necessary, EPA considers whether any ongoing testing will satisfy the information needs for the chemical and whether testing which the Agency might require would be capable of developing the necessary information.

EPA's approach to determining when these findings are appropriately made is described in detail in EPA's first and second proposed test rules as published in the **Federal Register** of July 18, 1980 (45 FR 48528) and June 5, 1981 (46 FR 30300).

For the finding under section 4(a)(1)(B)(i), EPA considers only production, exposure, and release information to determine if there is or may be substantial production and significant or substantial human exposure. For the findings under section 4(a)(1)(B)(ii), EPA examines toxicity and fate studies to determine if existing information is adequate to reasonably determine or predict the effects of human exposure to the chemical. In making the finding under section 4(a)(1)(B)(iii) that testing is necessary, EPA considers whether ongoing testing will satisfy the information needs for the chemical and whether testing which the Agency might require would be capable of developing the necessary information.

EPA's process for determining when these findings apply is described in detail in its second proposed test rule [See the **Federal Register** of June 5, 1981 (46 FR 30300)].

In evaluating ITC's testing recommendations for the three triethylene glycol ethers, EPA considered all available relevant information including the following: information presented in ITC's report recommending testing consideration; production volume, use, exposure, and release information reported by manufacturers of these compounds under TSCA section 8(a) Preliminary Assessment Information Rule (40 CFR Part 712); health and safety studies submitted under the TSCA section 8(d) Health and Safety Data Reporting Rule (40 CFR Part 716); and published and unpublished data available to the Agency. From its evaluation, as described in this proposed rule, EPA is proposing health effects testing requirements for triethylene glycol monomethyl, monoethyl, and monobutyl ethers under sections 4(a)(1)(A) and 4(a)(1)(B). By these actions, EPA is resposnding to ITC's designation of triethylene glycol monomethyl, monoethyl, and monobutyl; ethers for priority testing consideration.

II. Review of Available Data

A. Physicochemical Information

These three compounds are liquids, with very low estimated vapor pressures (Ref. 1).

Triethylene glycol monomethyl ether—2.90×10^{-3} mm Hg

Triethylene glycol monoethyl ether—2.90×10^{-3} mm Hg

Triethylene glycol monobutyl ether—2.50×10^{-3} mm Hg

B. Production and Use

These three chemicals are primarily co-produced during the manufacture of lower molecular weight glycol ethers. About 5 percent of the production is purified further for use as chemical intermediates, but the majority of production is sold in a technical grade for use as a diluent in brake fluids. The glycol ethers comprise 95–97 percent of the market for brake fluid diluents (Ecomomic Analysis Support Document). The International Trade Commission (1985) has estimated the 1984 production levels of these compounds to be as follows (Economic Analysis Support Document):

Triethylene glycol monomethyl ether—27 million lbs.

Triethylene glycol monoethyl ether—24 million lbs.

Triethylene glycol monobutyl ether—11 million lbs.

C. Exposure and Release

Preliminary data from the National Occupational Exposure Survey (NOES) conducted by the National Institute for Occupational Safety and Health (NIOSH) from 1980–1983 indicate that 248,333 workers, including 8,107 females, were potentially exposed to brake fluids in the workplace in 1980 (Ref. 2).

There are no data on levels of dermal exposure in the workplace or on release to the environment. However, the nature of brake system maintenance and repair suggests that complete exposure of both hands occurs regularly, even daily, for many professional mechanics. Furthermore, there is a potential for consumer exposure, since some individuals can be expected to add brake fluid or perform brake maintenance on their automobiles.

D. Health Effects

Acute toxicity tests have been done on all these chemicals by the oral, dermal and inhalation routes, although in different species. Oral and dermal median lethal doses (LD_{50}'s) for each compound are of the same order of magnitude, which indicates that the compound is dermally absorbed. While these studies differ in some respects from the corresponding TSCA test guidelines, EPA believes that the data are sufficient to predict the acute effects of these compounds, and that further acute studies need not be performed.

However, a complete health effects profile of these three triethylene glycol ethers is not established. Only triethylene glycol monoethyl ether has been tested in other than acute tests. Kondratyuk *et al.* (Ref. 3) gave a brief summary of the results of a 6-month gavage study in rats. Hypochromatic anemia, leucocytosis, eosinophilia, lymphocytopenia and monocytopenia, and liver and kidney dysfunction were seen at 0.775 and 7.75 mg/kg. A no-observed-effect level (NOEL) was seen at 0.0775 mg/kg. These data indicate a potential for chronic blood and organ effects for triethylene glycol monoethyl ether, as well as for the chemically related triethylene glycol monomethyl ether and triethylene glycol monobutyl ether, but the information available is insufficient for EPA to evaluate the possible risks to humans, as no specific histopathologic or biochemical data are provided.

Fetotoxicity and testicular atrophy have been noted with ethylene glycol monomethyl ether, a congener of triethylene glycol monomethyl ether (Ref. 4). Behavioral and neurochemical alterations have been seen in rats exposed *in utero* to the same chemical (Ref. 5), as well as encephalopathy in humans exposed in a work situation (Ref. 6). Another congener, diethylene glycol monobutyl ether, has given a positive response in the mammalian cells in culture gene mutation assay using mouse lymphoma cells (Ref. 7).

III. Findings

EPA is basing its proposed health effects testing of these glycol ethers on the authority of sections 4(a)(1)(A) and 4(a)(1)(B) of TSCA.

Under section 4(a)(1)(A) EPA finds that the use of the triethylene glycol ethers listed above may present an unreasonable risk of chronic toxicity based upon the chronic toxicity in the hematopoietic system and kidney and liver dysfunction seen with triethylene glycol monoethyl ether. The 4(a)(1)(A) findings for mutagenicity, developmental toxicity, neurotoxicity, developmental neurotoxicity and reproductive toxicity are based on positive results seen for related chemicals (Unit II.D).

Under section 4(a)(1)(B) of TSCA the Agency finds that triethylene glycol monomethyl, monoethyl, and monobutyl ethers are produced in substantial quantities and that there is substantial human exposure in the workplace as a result of the use of these substances in brake fluid.

The Agency also finds that the available data are insufficient to reasonably predict or determine the effects of the use of these compounds, and that testing is necessary to develop such data.

IV. Proposed Rule

A. Proposed Testing and Test Standards

The Agency is proposing that health effects testing be conducted on triethylene glycol monomethyl, monoethyl, and monobutyl ethers in accordance with specific guidelines set forth in Title 40 Part 798 published in the **Federal Register** of September 27, 1985 (50 FR 39252) as modified in the **Federal Register** of January 14, 1986 (51 FR 1522), as enumerated below. As all of these chemicals will be proposed for the same tests, the term "glycol ether" will refer to each of them as discussed below.

All the tests that can be performed by the dermal route are being proposed by that route because the expected human exposure is dermal. The rabbit has been proposed for the subchronic test because in studies done with related compounds, the rabbit was more sensitive to dermal exposure than the rat.

This proposed rule is a two-stage rule. The following tests will be incorporated in the first stage: subchronic toxicity, neurotoxicity, developmental toxicity and developmental neurotoxicity, and the lower-tier mutagenicity. The Agency will review all these data as received, decide which of the second-stage tests should be finalized, and publish a notice of that decision requesting public comment. EPA will hold a public meeting to discuss this decision if it is requested.

The second-stage tests may include the two-generation reproductive toxicity test, the heritable translocation test, the mouse specific locus test and the oncogenicity test.

All of the tests will be proposed at this time, but the final rule will include only the first-stage tests. The second-stage tests which EPA believes are needed following review of the first-stage tests will be made final following the public meeting and time for public comment.

Each glycol ether will be tested for subchronic toxicity, with special testing for liver dysfunction, kidney dysfunction, hematologic effects and reproductive effects. Exposure will be by the dermal route in the rabbit. Special organs of the reproductive tract to be weighed and evaluated are listed in § 799.4440. Urinalyses in all animals will be done before dosing begins, at day 30 and day 90 in order to examine kidney function. The details for the liver dysfunction tests and the special hematologic studies are given in § 799.4440. Subchronic dermal neurotoxicity studies will be performed in the rat: A functional observational battery (section 798.6050), motor activity (section 798.6200), and neuropathology (section 798.6400). These neurotoxicity tests may be combined, using 10 animals for each dose and sex.

To assess the potential for gene mutations, the Agency is proposing mutagenicity testing in the *Salmonella* reverse mutation assay as specified in § 798.5265 for all three glycol ethers. In each case, if the *Salmonella* result is negative, a mammalian cells in culture test shall be done (section 798.5300). If the mammalian cells in culture test is negative, no further gene mutation studies need be done.

If either the *Salmonella* or mammalian cells in culture test is non-negative, a *Drosophila* sex-linked recessive lethal test (section 798.5275) shall be performed for the chemical. If the sex-linked recessive lethal test is negative, no further gene mutation studies need be done. If the *Drosophila* test is positive, EPA will consider requiring a mouse visible specific locus test (section 798.5200) in the second-stage rule.

For testing for chromosomal aberrations, each of the glycol ethers shall undergo a tiered testing scheme. The first test is the *in vitro* cytogenetic chromosomal aberration test (section 798.5375). If this test is negative, the chemical shall be tested in the *in vivo* cytrogenetic assay (section 798.5385). If this test is also negative, no further testing for chromosomal effects need be done. If either the *in vitro* or *in vivo* test is non-negative for any chemical, then a dominant lethal study in the rat shall be performed (section 798.5450). If the dominant lethal study is negative, no further chromosomal aberration studies need be done, and if the dominant lethal test is positive, then EPA will consider requiring a mouse heritable translocation test (§ 798.5460) in the second stage final rule.

EPA is requiring developmental toxicity testing in the rabbit and the rat, according to § 798.4900. This study shall be by the dermal route of exposure. Another dermal rat developmental neurotoxicity study, according to § 795.250 shall be performed in which the offspring shall be allowed to go to parturition, and those offspring shall be evaluated for behavioral alterations at various stages following birth. The developmental neurotoxicity test shall be performed after the developmental toxicity study has been done in order to determine appropriate doses, i.e., the developmental neurotoxicity study shall be performed at doses lower than those

which induce severe teratogenic or fetal effects.

The Agency is proposing that an oral two-generation reproductive test (section 798.4700) be required in the rat if the results of gross or histopathologic evaluation of the reproductive tissue in male or female exposed rabbits from the subchronic exposure test show adverse effects. Following the completion of the subchronic study, a public review of the data will be held to determine if the Agency should promulgate a final rule requiring a two-generation study.

If either the Drosophila sex-linked recessive lethal or the dominant lethal test for any of these compounds is positive, a public program review will be held before the final tier testing for mutagenicity of that compound is required.

Oncogenicity studies (section 798.3300) for each of these three chemicals may be required in the mouse and rat by dermal absorption. EPA will review the mutagenicity data and all other available data related to oncogenicity and hold a public program review before publishing a final rule on oncogenicity.

B. Test Substance

EPA is proposing testing of the triethylene glycol ethers of at least 90-percent purity. The EPA believes that test materials of this purity are available at reasonable cost. The Agency has specified relatively pure substances for testing because it is interested in evaluating the effects attributable to the subject compounds themselves. This requirement would lessen the likelihood that any effects seen are due to impurities.

C. Persons Required to Test

Section 4(b)(3)(B) of TSCA specifies that the activities for which the Agency makes section 4(a) findings (manufacture, processing, distribution, use and/or disposal) determine who bears the responsibility for testing. Manufacturers are required to test if the findings are based on manufacturing, which includes production of these chemicals as a co-product ("manufacture" is defined in section 3(7) of TSCA to include "import"). Processors are required to test if the findings are based on processing. Both manufacturers and processors are required to test if the exposures causing the potential risk occur during use, distribution, or disposal.

Because EPA has found that existing data are inadequate to assess the health risks from the use of these compounds, the EPA is proposing that persons who manufacture and/or process, or who intend to manufacture and/or process, these glycol ethers at any time from the effective date of the final test rule to the end of the reimbursement period be subject to the testing requirements in this proposed rule. The end of the reimbursement period will be 5 years after the last final report is submitted or an amount of time equal to that which was required to develop data, if more than 5 years, after the submission of the last final report required under the test rule.

Because TSCA contains provisions to avoid duplicative testing, not every person subject to this rule must individually conduct testing. Section 4(b)(3)(A) of TSCA provides that EPA may permit two or more manufacturers or processors who are subject to the rule to designate one such person or a qualified third person to conduct the tests and submit data on their behalf. Section 4(c) provides that any person required to test may apply to EPA for an exemption from the requirement. EPA promulgated procedures for applying for TSCA section 4(c) exemptions in 40 CFR Part 790.

Manufacturers (including importers) subject to this rule are required to submit either a letter of intent to perform testing or an exemption application within 30 days after the effective date of the final test rule. The required procedures for submitting such letters and applications are described in 40 CFR Part 790.

Processors subject to this rule, unless they are also manufacturers, will not be required to submit letters of intent or exemption applications, or to conduct testing, unless manufacturers fail to submit notices of intent to test or later fail to sponsor the required tests. The Agency expects that the manufacturers will pass an appropriate portion of the costs of testing on to processors through the pricing of their products or reimbursement mechanisms. If manufacturers perform all the required tests, processors will be granted exemptions automatically. If manufacturers fail to submit notices of intent to test or fail to sponsor all the required tests, the Agency will publish a separate notice in the Federal Register to notify processors to respond; this procedure is described on 40 CFR Part 790.

EPA is not proposing to require the submission of equivalence data as a condition for exemption from the proposed testing for these glycol ethers. As noted in Unit IV.B. of this preamble, the EPA is interested in evaluating the effects attributable to the specified compounds and has proposed relatively pure substances for testing.

Manufacturers and processors subject to this test rule must comply with the test rule development and exemption procedures in 40 CFR Part 790 for single-phase rulemaking.

D. Reporting Requirements

EPA is proposing that all data developed under this rule be reported in accordance with its TSCA Good Laboratory Practice (GLP) standards, which appear in 40 CFR Part 792.

In accordance with 40 CFR Part 790 under single-phase rulemaking procedures, test sponsors are required to submit individual study plans at least 45 days before the start of each study.

EPA is required by TSCA section 4(b)(1)(C) to specify the time period during which persons subject to a test rule must submit test data. The Agency is proposing specific reporting requirements for each of the proposed tests as follows:

1. The subchronic toxicity and subchronic neurotoxicity tests shall be completed and the final results submitted to the Agency within 15 months of the effective date of the final test rule.

2. The lower-tier mutagenicity studies shall be completed and final results submitted to the Agency within the deadlines specified in the rule. These range from 4 months after the effective date of the final test rule for the *in vitro* cytogenetics assay to 24 months for the *Drosophila* sex-linked recessive lethal assay and the dominant lethal assay.

3. The upper-tier mutagenicity tests shall be completed and final results submitted to the Agency within 12 months of the effective date of a final test rule requiring these studies.

4. The developmental toxicity studies shall be completed and final results submitted to the Agency within 12 months of the effective date of the final test rule. The developmental neurotoxicity test shall be completed and final results submitted to the Agency within 24 months of the effective date of the final test rule.

5. The oncogenicity tests shall be completed and the final results submitted to the Agency within 53 months of the effective date of a final test rule requiring this study.

6. The two-generation reproductive study shall be completed and the final results submitted to the Agency within 29 months of the effective date of a final test rule requiring that study.

Progress reports on these tests will be required at 6-month intervals beginning 6 months from the effective date of the final rule requiring that study.

TSCA section 14(b) governs Agency disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by this rule, the Agency will publish a notice of receipt in the **Federal Register** as required by section 4(d).

Persons who export a chemical substance or mixture which is subject to a section 4 test rule are subject to the export reporting requirements of section 12(b) of TSCA. Final regulations interpreting the requirements of section 12(b) are in 40 CFR Part 707. In brief, as of the effective date of this test rule, an exporter of any of the three triethylene glycol ethers referred to in this rule must report to EPA the first annual export or intended export of the compound to any one country. EPA will notify the foreign country about the test rule for the chemical.

E. Enforcement Provisions

The Agency considers failure to comply with any aspect of a section 4 rule to be a violation of section 15 of TSCA. Section 15(1) of TSCA makes it unlawful for any person to fail or refuse to comply with any rule or order issued under section 4. Section 15(3) of TSCA makes it unlawful for any person to fail or refuse to: (1) establish or maintain records (2) submit reports, notices, or other information, or (3) permit access to or copying of records required by the Act or any regulation or rule issued under TSCA.

Additionally, TSCA section 15(4) makes it unlawful for any person to fail or refuse to permit entry or inspection as required by section 11. Section 11 applies to any "establishment, facility, or other premises in which chemical substances or mixtures are manufactured, processed, stored, or held before or after their distribution in commerce * * *." The Agency considers a testing facility to be a place where the chemical is held or stored and, therefore, subject to inspection. Laboratory inspections and data audits will be conducted periodically in accordance with the authority and procedures outlined in TSCA section 11 by duly designated EPA representatives to determine compliance with any final rule for these glycol ethers. These inspections may be conducted for purposes which include verification that testing has begun, that schedules are being met, and that reports accurately reflect the underlying raw data and interpretations and evaluations to determine compliance with TSCA GLP standards and the test standards established in the rule.

EPA's authority to inspect a testing facility also derives from section 4(b)(1) of the TSCA, which directs EPA to promulgate standards for the development of test data. These standards are defined in section 3(12)(B) of TSCA to include those requirements necessary to assure that data developed under testing rules are reliable and adequate, and such other requirements as are necessary to provide such assurance. The Agency maintains that laboratory inspections are necessary to provide this assurance.

Violators of TSCA are subject to criminal and civil liability. Persons who submit materially misleading or false information in connection with the requirement of any provision of this rule may be subject to penalties which may be calculated as if they never submitted their data. Under the penalty provision of section 16 of TSCA, any person who violates section 15 could be subject to a civil penalty of up to $25,000 for each violation with each day of operation in violation constituting a separate violation. This provision would be applicable primarily to manufacturers or processors that fail to submit a letter of intent or an exemption request and that continue manufacturing or processing after the deadlines for such submissions. This provision would also apply to processors that fail to submit a letter of intent or an exemption application and continue processing after the Agency has notified them of their obligation to submit such documents (see 40 CFR 790.28(b)). Intentional violations could lead to theimposition of criminal penalties of up to $25,000 for each day of violation and imprisonment for up to 1 year. In determining the amount of penalty, EPA will take into account the seriousness of the violation and the degree of culpability of the violator as well as all the other factors listed in section 16. Other remedies are available to EPA under section 17 of TSCA, such as seeking an injunction to restrain violations of TSCA section 4.

Individuals as well as corporations could be subject to enforcement actions. Sections 15 and 16 of TSCA apply to "any person" who violates various provisions of TSCA. At its discretion, EPA may proceed against individuals as well as companies. In particular, this includes individuals who report false information or who cause it to be reported. In addition, the submission of false, fictitious, or fraudulent statements is a violation under 18 U.S.C.1001.

V. Issues for Comment

1. EPA is proposing the rat as the species in which to perform an oral two-generation reproductive toxicity study, although the normal human exposure is dermal. Should the reproductive study be done dermally? The rabbit has proved to be more sensitive to the effects of related glycol ethers than the rat when treated dermally. However, some persons feel that the rabbit is inappropriate for doing two-generation reproductive toxicity studies, either dermally or orally, because rabbits do not breed well in captivity. The Agency invites comment on these issues.

2. EPA is proposing that the *in vivo* cytogenetics and the dominant lethal mutagenicity tests be done by the dermal route because humans are expected to be exposed dermally. However, mutagenicity testing by the dermal route has little precedent. Furthermore, the mouse specific locus test and the heritable translocation test are being proposed by the oral route because no historical controls are available for the dermal route. The Agency invites comment on the desirability of dermal administration of the glycol ethers in the mutagenicity tests.

3. This test rule may result in three oncogenicity tests, one for each glycol ether being tested. Is it appropriate to consider in that case requiring oncogenicity testing for only the most potent chemical as determined from the first-stage mutagenicity testing to share the costs and reduce the economic impact, and regulating all three on that basis? Should this approach be considered for the other stage-two tests proposed in this rule?

4. Although the Level I economic analysis indicates a potential for significant economic impact (see Unit VI), EPA will not be performing a Level II economic analysis until after proposal of this rule. In order to refine the economic analysis, EPA requests comments on the economic impact on the manufacturers, processors and users.

5. EPA is proposing in this notice a two-stage test rule. The first stage will include the subchronic toxicity test, the developmental toxicity tests, neurotoxicity tests and the lower-tier mutagenicity tests. EPA will review the data after it is received, approximately two years from the final rule, and will then hold a public review meeting to discuss EPA's decisions as to which of the second-stage tests are necessary. The second-stage testing includes oncogenicity, mouse specific locus, heritable translocation and two-generation reproductive toxicity tests. The Agency believes that this approach provides needed flexibility in dealing with this group of chemicals and requests comment from interested parties.

6. EPA is proposing extensive testicular histopathology in the 90-day subchronic, because members of this class of compounds are known to cause testicular atrophy. The effects seen have been described in detail and the modifications proposed in this notice specifically address these concerns. However, the chemicals tested for reproductive toxicity have affected the female as much as the male in some cases. Because it is more difficult to predict reproductive effects from standardized subchronic histopathology on the female reproductive organs, EPA has not routinely used such testing to trigger a two-generation reproductive test. However, for these glycol ethers, EPA is considering requiring data on the estrous cycle in exposed animals by performing vaginal cytology over the last two weeks of exposure in the 90-day subchronic (Ref. 8), and more detailed histopathologic analysis of the ovary to evaluate oocyte toxicity (Ref. 9). The Agency requests comments on whether EPA should require the cyclicity study and the detailed female histopathology, and on the availability of test facilities with experience in such studies.

7. It has been suggested that the rats in the subchronic neurotoxicity tests also be examined for reproductive effects. Most of the available research has been performed in the rat, which would make the results easier to evaluate. In addition, EPA could require a satellite group for reproduction and fertility study. The Agency requests comments on the usefulness of these proposed protocol changes to predict reproductive toxicity.

8. EPA is proposing that the developmental neurotoxicity screen be performed using the dermal route of exposure. Because exposure of the dams will continue through lactation, dermal administration to the dams may result in the pups being exposed directly to the compound, either dermally or orally, instead of just indirectly through the milk. Should this study be performed using the oral route?

9. It has further been suggested that the oral route be used for all the tests. The reason for proposing the dermal route has been discussed earlier (see Unit IV.A. and Issues 1. and 2.). Dermal and oral disposition and metabolism tests may give the Agency the option to require oral testing and use the comparative oral and dermal chemical disposition and metabolism testing to estimate the appropriate dermal dose an individual might receive. Should the Agency evaluate this option?

10. If this option is considered, what are the pros and cons of using an *in vitro* percutaneous absorption study rather than an *in vivo*? Using an *in vitro* study would allow the tester to use human skin to measure absorption which may more effectively simulate human exposure. However, there is some indication that using skin from different individuals may result in widely varying figures, whereas using skin samples from an in-bred rat strain gives very reproducible results.

11. Recently the Chemical Manufacturers Association (CMA) informed EPA about three studies it is sponsoring on these triethylene glycol ethers, a 14-day dermal limit test, an *in vitro* percutaneous absorption study, and a Chernoff-Kavlock teratogenicity bioassay. The Agency is requesting comments on the use of the Chernoff-Kavlock assay specifically for the glycol ether chemical class as a screen for further testing needs, or as a replacement for one of the species in the standard developmental toxicity assay.

12. The Agency prefers to require testing of commercially available substances of the highest purity, rather than proposing that the manufacturers purify the compounds to a pre-designated level. However, if the impurities in the triethylene glycol ethers are the monoethylene congeners, which are known toxicants, it is possible that the perceived toxicity of the test substances might be increased. Should the EPA require in this case that a greater than commercial purity be achieved, or that no other glycol ethers be found as impurities in the test substance?

VI. Economic Analysis of Proposed Rule

To evaluate the potential economic impact of test rules, EPA has adopted a two-stage approach. All candidates for test rules go through a Level I analysis. This consists of evaluating each chemical or chemical group on four principal market characteristics: (1) Demand sensitivity, (2) cost characteristics, (3) industry structure, and (4) market expectations. The results of the Level I analysis, along with the consideration of the costs of the required tests, indicate whether the possibility of a significant adverse economic impact exists. Where the indication is negative, no further economic analysis is done for the chemical substance or group. However, for those chemical substances or groups where the Level I analysis indicates a potential for significant economic impact, a more comprehensive and detailed analysis is conducted. This Level II analysis attempts to predict more precisely the magnitude of the expected impact.

Total testing costs for the proposed rule for each triethylene glycol ether are estimated to range from $1,292,008 to $1,838,190. This estimate includes the costs for the required minimum series of tests as well as the conditional ones. The annualized test costs (using a cost of capital of 25 percent over a 15-year product life) range from $334,761 to $476,306 for each chemical. When broken down into the two stages proposed in this rule, the total testing costs for the first stage are estimated to range from $318,948 to $495,960, and the annualized test costs from $82,645 to $128,512. The total testing costs for the second stage are estimated to range from $973,060 to $1,342,230 and the annualized test costs from $252,136 to $347,794. Based on the estimated 1984 production volumes listed in Unit II.A., the unit test costs, the average selling price and the relative costs are listed below for each glycol ether.

For the first stage testing for each chemical:

Triethylene glycol monomethyl ether: unit test cost 0.48 cent per pound, average selling price $0.44 per pound, relative cost 1.08 percent of price.

Triethylene glycol monoethyl ether: unit test cost 0.54 cent per pound, average selling price $0.50 per pound, relative cost 1.07 percent of price.

Triethylene glycol monobutyl ether: unit test cost 1.2 cents per pound, average selling price $0.43 per pound, relative cost 2.72 percent of price.

For the total testing for each chemical:

Triethylene glycol monomethyl ether: unit test cost 1.8 cents per pound, average selling price $0.44 per pound, relative cost 4.01 percent of price.

Triethylene glycol monoethyl ether: unit test cost 2.0 cents per pound, average selling price $0.50 per pound, relative cost 3.97 percent of price.

Triethylene glycol monobutyl ether: unit test cost 4.3 cents per pound, average selling price $0.43 per pound, relative cost 10.07 percent of price.

The two-stage testing proposal has mitigated the economic impact for all three chemicals to some extent, so that a potential for significant economic impact is not expected for triethylene glycol monomethyl ether and triethylene glycol monoethyl ether for the first-stage testing. However, a Level II economic analysis will be done on triethylene glycol monobutyl ether after receiving comments on this proposal. Following completion of the first-stage testing, EPA will evaluate the results and decide whether the importance of the potential adverse health effects outweighs the possible adverse economic effects,

before requiring the second stage of testing.

VII. Availability of Test Facilities and Personnel

Section 4(b)(1) of TSCA requires EPA to consider "the reasonably foreseeable availability of the facilities and personnel needed to perform the testing required under the rule". Therefore, EPA conducted a study to assess the availability of test facilities and personnel to handle the additional demand for testing services created by section 4 test rules. Copies of the study, Chemical Testing Industry: Profile of Toxicological Testing, can be obtained through the NTIS (PB 82-140773). On the basis of this study, the Agency believes that there will be available test facilities and personnel to perform the testing specified in this proposed rule.

VIII. Public Meetings

If persons indicate to EPA that they wish to present oral comments on this proposed rule to EPA officials who are directly responsible for developing the rule and supporting analyses, EPA will hold a public meeting after the close of the public comment period in Washington, DC. Persons who wish to attend or to present comments at the meeting should call the TSCA Assistance Office (TAO): Toll Free: (800—424-9065); in Washington, DC: (554-1404); Outside the U.S.A. (Operator—202-554-1404), by June 30, 1986. A meeting will not be held if members of the public do not indicate that they wish to make oral presentations. While the meeting will be open to the public, active participation will be limited to those persons who arranged to present comments and to designated EPA participants. Attendees should call the TAO before making travel plans to verify whether a meeting will be held.

Should a meeting be held, the Agency will transcribe it and include the written transcript in the public record. Participants are invited, but not required, to submit copies of their statements prior to or on the day of the meeting. All such written materials will become part of EPA's record for this rulemaking.

IX. Public Record

EPA has established a record for this rulemaking, (docket number OPTS-42080). This record contains the basic information considered by the Agency in developing this proposal and appropriate Federal Register notices.

This record includes the following information:

A. Supporting Documentation

(1) Federal Register notices pertaining to this rule consisting of:

(a) Notice containing the ITC designation of triethylene glycol monomethyl, monoethyl, and monobutyl ethers.

(b) Rules requiring TSCA section 8 (a) and (d) reporting on triethylene glycol monomethyl, monoethyl, and monobutyl ethers.

(c) Notice of final rule on EPA's TSCA good laboratory practice standards (48 FR 53922; November 29, 1983).

(d) Notice of interim final rule on single-phase test rule development and exemption procedures (50 FR 20652; May 17, 1985).

(e) Notice of final rule on data reimbursement policy and procedures (48 FR 31786; July 11, 1983).

(2) Support document consisting of triethylene glycol monomethyl, monethyl, and monobutyl ethers' economic analysis.

(3) TSCA test guidelines and other test methodologies cited as test standards for this rule (50 FR 39252; September 27, 1985; 51 FR 1522; January 14, 1986.)

(4) Communications before proposal consisting of:

(a) Written public comments and letters.

(b) Contact reports of telephone conversations.

(c) Meeting summaries.

(5) Reports—published and unpublished factual materials.

B. References

(1) USEPA. U.S. Environmental Protection Agency. Memorandum from Patricia Harrigan to Gary Timm on Chemical Property and Environmental Behavior Estimates for Chemicals on the 16th ITC Priority List. Office of Toxic Substances (June 7, 1985).

(2) NIOSH. National Occupational Exposure Survey (1980-1983). Cincinnati, OH: Department of Health and Human Services, National Institute for Occupational Safety and Health. (1985).

(3) Kondratyuk, V.A., Pis'ko, G.T., Sergema, V.N., Gum'ko, L.M., Fira, L.S., Pastushenko, T.V. *et al.* "Establishment of the maximum permissible concentration of triethylene glycol ethyl ether in reservoir water." *Gigiena Sanitariya* 5:84-85 (1982).

(4) Doe, J.E. "Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment." *Environmental Health Perspectives* 57:199-206 (1984).

(5) Nelson, B.K., Brightwell, W.S., Burg, J.R., and Massari, V.J. "Behavioral and neurochemical alterations in the offspring of rats after maternal or paternal inhalation exposure to the industrial solvent 2-methoxyethanol." *Pharmacology, Biochemistry and Behavior* 20:269-279 (1984).

(6) Ohi, G. and Wegman, D.H. "Transcutaneous ethylene glycol monomethyl ether poisoning in the work setting." *Journal of Occupational Medicine* 20(10):675-676 (1978).

(7) Thompson, E.D., Coppinger, W.J., Valencia, R. and Iavicoli, J. "Mutagenicity testing of diethylene glycol monobutyl ether." *Environmental Health Perspectives* 57:105-112 (1984).

(8) Sadleir, R.M.F.S. "Cycles and seasons." *Reproduction in Mammals: I. Germ Cells and Fertilization.* Ed. Austin, C.R., Short, R.V., New York: Cambridge Press, pp. 85-102 (1978).

(9) Mattison, D.R. "Morphology of oocyte and follicle destruction by polycyclic aromatic hydrocarbons in mice." *Toxicology and Applied Pharmacology* 53:249-259 (1980).

Confidential Business Information (CBI), while part of the record, is not available for public review. A public version of the record, from which CBI has been deleted, is available for inspection in the OPTS Reading Rm. E-107, 401 M St., SW., Washington, DC, from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

X. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA must judge whether a regulation is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. EPA has determined that this test rule is not major because it does not meet any of the criteria set forth in section 1(b) of the Order, i.e., it will not have an annual effect on the economy of at least $100 million, will not cause a major increase in prices, and will not have a significant adverse effect on competition or the ability of U.S. enterprises to compete with foreign enterprises.

This proposed regulation was submitted to the Office of Management and Budget (OMB) for review as required by Executive Order 12291. Any written comments from OMB to EPA, and any EPA response to those comments, are included in the rulemaking record.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (15 U.S.C. 601 *et seq.*, Pub. L. 96-354, September 19, 1980), EPA is certifying that this test rule, if promulgated, will not have a significant impact on a substantial number of small businesses because: (1) They are not expected to perform testing themselves, or to participate in the organization of the testing effort; (2) they will experience only very minor costs in securing exemption from testing requirements; and (3) they are unlikely to be affected by reimbursement requirements.

C. Paperwork Reduction Act

The information collection requirements contained in this rule have been approved by OMB under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and have been assigned OMB number 2070-

0033. Comments on these requirements should be submitted to the Office of Information and Regulatory Affairs of OMB; 726 Jackson Place, NW., Washington, DC 20503 marked "Attention: Desk Officer for the EPA". The final rule package will respond to any OMB or public comments on the information collection requirements.

List of Subjects in 40 CFR Parts 795 and 799

Testing, Environmental protection, Hazardous substances, Chemicals, Recordkeeping and reporting requirements.

Dated: May 2, 1986.

Victor J. Kimm,

Deputy Assistant Administrator for Pesticides and Toxic Substances.

Therefore, it is proposed that 40 CFR Chapter I be amended as follows:

PART 795—[AMENDED]

1. In proposed Part 795:

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. New § 795.250 is added to read as follows:

§ 795.250 Developmental neurotoxicity screen.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, it is important to determine when acceptable exposures in the adult may not be acceptable to a developing organism. This study is designed to provide information on the potential functional and morphologic hazards to the nervous system which may arise in the offspring from exposure of the mother during pregnancy and lactation. If effects are detected, further studies may be required to characterize and assess the risk(s).

(b) *Principle of the test method.* The test substance is administered to several groups of pregnant animals during gestation and lactation, one dose level being used per group. Offspring are randomly selected from within litters for neurotoxicity evaluation. The evaluation includes observation to detect gross neurologic and behavioral abnormalities, determination of motor activity, neurophathological evaluation, and brain weight. Measurements are carried out periodically during both postnatal development and adulthood.

(c) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Testing shall be performed in the rat.

(ii) *Age.* Young adult animals (nulliparous females) shall be used.

(iii) *Sex.* Pregnant animals shall be used at each dose level.

(iv) *Number of animals.* A sufficient number of pregnant rats shall be exposed to ensure that an adequate number of offspring are produced for neurotoxicity evaluation. Sample size should be based on the test requiring the greatest number of offspring to achieve sensitivity. The test should be able to detect a 20 percent difference in the test group relative to the control group with 90 percent power at the 5 percent level. For most designs, calculations can be made according to Dixon and Massey (1957) under paragraph (e)(3) of this section, Neter and Wasserman (1974) under paragraph (e)(7) of this section, Sokal and Rohlf (1969) under paragraph (e)(8) of this section, or Jensen (1972) under paragraph (e)(5) of this section. The size of each litter shall be adjusted as outlined in the Toxic Substances Control Act (TSCA) reproduction and fertility effects guideline, 40 CFR 798.4700, as published in the Federal Register of September 27, 1985 (50 FR 39432). One male and one female shall be randomly selected from each litter for interim sacrifice at weaning. One male and one female shall be randomly selected from each litter for behavioral assessment and terminal sacrifice. It is also recommended that additional males and females randomly selected from each litter be assigned to different tasks to eliminate any confounding from multiple testing. If the behavioral tasks are conducted in the same animal, then the sequence should be locomotor activity, auditory startle, maze performance. A minimum of 1–2 days should separate each test.

(2) *Control group.* A concurrent control group shall be used. This group shall be a sham treated control group, or, if a vehicle is used in administering the test substance, a vehicle control group. Animals in the control group(s) shall be handled in an identical manner to test group animals. The vehicle shall neither be developmentally toxic nor have effects on reproduction.

(3) *Dose levels and dose selection.* (i) At least 3 dose levels with a control (vehicle control, if vehicle is used) shall be used.

(ii) If the substance has been shown to be developmentally toxic, either in a standard developmental toxicity study or a pilot study, the highest dose level shall be the maximum dose which will not induce *in utero* or neonatal death or malformations sufficient to preclude a meaningful evaluation of neurotoxicity.

(iii) In the absence of standard developmental toxicity, unless limited by the physicochemical nature or biological properties of the substance, the highest dose level shall induce some overt maternal toxicity such as a 20 percent reduction in weight gain throughout gestation and lactation.

(iv) The lowest dose level should not produce any grossly observable evidence of either maternal or developmental neurotoxicity.

(v) The intermediate dose(s) shall be equally spaced between the highest and lowest dose, on a log scale.

(4) *Dosing period.* Day 0 in the test is the day on which a vaginal plug and/or sperm are observed. The dose period shall cover the period from Day 6 of gestation through weaning (21 days).

(5) *Administration of test substance.* The test substance or vehicle is usually administered orally, by intubation, unless the chemical or physical characteristics of the test substance or pattern of human exposure suggest a more appropriate route of administration. The test substance shall be administered at the same time each day. The animals shall be weighed periodically and the dosage based on the most recent weight determination.

(6) *Observation of dams.* (i) A gross examination of the dams shall be made at least once each day, before daily treatment. The animals shall be observed each day by the same trained technician, who shall be blind with respect to the animals' treatment.

(ii) During the treatment and observation periods, cage-side observations shall include:

(A) Any unusual responses with respect to body position, activity level, coordination of movement, and gait.

(B) Any unusual or bizarre behavior including, but not limited to, headflicking, headsearching, compulsive biting or licking, self-mutilation, circling, and walking backwards.

(C) The presence of:

(*1*) Convulsions;

(*2*) Tremors;

(*3*) Increased levels of lacrimation and/or red-colored tears;

(*4*) Increased levels of salivation;

(*5*) Piloerection;

(*6*) Pupillary dilation or constriction;

(*7*) Unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing;

(*8*) Diarrhea;

(*9*) Excessive or diminished urination;

(*10*) Vocalization.

(iii) Signs of toxicity shall be recorded as they are observed, including the time of onset, the degree and duration.

(iv) Animals shall be weighed at least weekly.

(7) *Study conduct.* (i) Physical landmarks of development. Offspring shall be weighed at birth, days 12, 17, 21, and biweekly thereafter. The age of the

following physical landmarks shall be determined:

(A) Eye opening;
(B) Incisor eruption;
(C) Vaginal opening;
(D) Testes descent.

General procedures for these determinations may be found in Adams *et al.* (1985) under paragraph (e)(1) of this section.

(ii) Motor activity shall be monitored on days 13, 17, 21, 30, 45, and 60. Motor activity must be monitored by an automated activity recording apparatus. The device used must be capable of detecting both increases and decreases in activity, i.e., baseline activity as measured by the device must not be so low as to preclude decreases nor so high as to preclude increases. Each device shall be tested by standard procedure to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices.

(A) Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for most treatments and animals' activity counts shall be collected in equal time periods of no greater than 10 minutes duration. All sessions shall have the same duration. Treatment groups shall be counter-balanced across test times.

(B) Efforts shall be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables which can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, lighting conditions, odors, use of home cage or novel test cage, and environmental distractions. Tests shall be executed by an appropriately trained individual.

(C) Additional information on the conduct of a motor activity study may be obtained in the TSCA motor activity guideline, 40 CFR 798.6200, as published in the Federal Register of September 27, 1985 (50 FR 39460).

(iii) Observation of offspring. (A) The offspring shall be examined cage-side daily for gross signs of mortality and morbidity.

(B) The offspring shall be examined outside the cage for gross signs of toxicity whenever they are weighed or removed from their cages for behavioral testing. As a minimum, the endpoints outlined in paragraph (6)(ii) shall be used.

(C) Any gross signs of toxicity in the offspring shall be recorded as they are observed, including the time of onset, the degree and duration.

(iv) An auditory startle habituation test shall be performed on the offspring on days 22 and 60. Details on the conduct of this testing may be obtained in Kellog *et al.* (1980) under paragraph (e)(6) of this section and Adams *et al.* (1985) under paragraph (e)(1) of this section.

(v) The Biel water maze paradigm shall be conducted beginning at approximately day 55 of age. Details on the conduct of this testing may be obtained in Vorhees *et al.* (1978) under paragraph (e)(11) of this section.

(B) *Post-mortem evaluation*—(i) *Age of animals.* One male and one female per litter shall be sacrificed at weaning and the remainder following the last behavioral measures. Both neuropathology and brain weight determinations shall be made at both time points.

(ii) *Neuropathology.* Details for the conduct of neuropathology may be obtained in 40 CFR 798.6400, as published in the Federal Register of September 27, 1985 (50 FR 39461). At least 6 offspring per dose group shall be randomly selected from each sacrificed group (weaning and adulthood) for neuropathologic evaluation. These animals shall be balanced across litters and equal numbers of males and females shall be used. The remaining sacrificed animals shall be used to determine brain weight. Animals shall be perfused *in situ* by a generally recognized technique. After perfusion, the brain and spinal cord shall be removed and gross abnormalities noted. Cross-sections of the following areas shall be examined: The forebrain, the center of the cerebrum and midbrain, the cerebellum and pons, and the medulla oblongata; the spinal cord at cervical and lumbar swelling; Gasserian ganglia, dorsal root ganglia, dorsal and ventral root fibers, proximal sciatic nerve (mid-thigh and sciatic notch), sural nerve (at knee), and tibial nerve (at knee). Tissue samples from both the central and peripheral nervous system shall be further immersion-fixed and stored in appropriate fixative for further examination. After dehydration, tissue specimens shall be cleared with xylene and embedded in paraffin or paraplast. Tissue sections shall be prepared from the tissue blocks. The following general testing sequence is proposed for gathering histopathological data:

(A) *General staining.* A general staining procedure shall be performed on all tissue specimens in the highest treatment group. Hematoxylin and eosin (H&E) shall be used for this purpose. The staining shall be differentiated properly to achieve bluish nuclei with pinkish background.

(B) *Special stains.* Based on the results of the general staining, selected sites and cellular components shall be further evaluated by the use of specific techniques. If H&E screening does not provide such information, a battery of stains shall be used to assess the following components in all appropriate required samples: neuronal body (e.g., Einarson's gallocyanin), axon (e.g., Kluver's Luxol Fast Blue) and neurofibrils (e.g., Bielchosky). In addition, nerve fiber teasing shall be used. A section of normal tissue shall be included in each staining to assure that adequate staining has occurred. Any changes shall be noted and representative photographs shall be taken. If a lesion(s) is observed, the special techniques shall be repeated in the next lower treatment group until no further lesion is detectable.

(C) *Alternative technique.* If the anatomical locus of expected neuropathology is well-defined, epoxy-embedded sections stained with toluidine blue may be used for small sized tissue samples. This technique obviates the need for special stains.

(iii) *Brain weight.* Animals that are not sacrificed for histopathology shall be used to determine brain weight. The animals shall be decapitated and the brains carefully removed, blotted, chilled and weighed. The following dissection shall be performed on an ice-cooled glass plate: First the rhombencephalon is separated by a transverse section from the rest of the brain and into the cerebellum and the medulla oblongata/pons. A transverse section is made at the level of the 'optic chiasma' which delimits the anterior part of the hypothalamus and passes through the anterior commissure. The cortex is peeled from the posterior section and added to the anterior section. This divides the brain into four sections, the telencephalon, the diencephalon/mid-brain, the medulla oblongata+pons, and the cerebellum. Sections shall be weighed as soon as possible after dissection to avoid drying. Detailed methodology is available in Glowinski and Iversen (1966) under paragraph (e)(4) of this section.

(d) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR Part 792, Subpart J, the final test report must include the following information.

(1) *Description of system and test methods.* (i) A detailed description of the procedures used to standardize observation and operational definitions for scoring observation.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. These data do not have to be from studies using prenatal exposures. However, the laboratory must demonstrate competence in testing neonatal animals perinatally exposed to chemicals and establish test norms for the appropriate age group.

(iii) Procedures for calibrating and assuring the equivalence of devices and balancing treatment groups.

(iv) A short justification explaining any decisions where professional judgement is involved such as fixation technique and choice of stains.

(2) *Results.* The following information must be arranged by test group dose level.

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number and litter from which it came.

(B) Its body weight and score on each sign at each observation time; total session activity counts; intrasession subtotals for each date measured; time and cause of death (if appropriate); location(s), nature of, frequency, and severity of the lesion(s); total brain weight; absolute weight of each of four sections; and weight of each section as a percentage of total brain weight. A commonly used scale such as 1+, 2+, 3+, and 4+ for degree of severity of lesions ranging from very slight to extensive may be used for morphologic evaluation. Any diagnoses derived from neurologic signs and lesions, including naturally occurring diseases or conditions, shall also be recorded.

(ii) Summary data for each group must include:

(A) The number of animals at the start of the test.

(B) Body weights of the dams during gestation and lactation.

(C) Litter size and mean weight at birth.

(D) The number of animals showing each observation score at each observation time.

(E) The percentage of animals showing each abnormal sign at each observation time.

(F) The mean and standard deviation for each continuous endpoint at each observation time. These will include body, weight, motor activity counts, acoustic startle responses, maze performance, and brain weights (both absolute and relative).

(G) The number of animals in which any lesion was found.

(H) The number of animals affected by each different type of lesion, the average grade of each type of lesion and the frequency of each different type and/or location of lesion.

(3) *Evaluation of data.* An evaluation of the test results must be made. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence, and severity of any neurotoxic effects. The evaluation shall include appropriate statistical analyses. The choice of analyses shall consider tests appropriate to the experimental design and needed adjustments for mulitple comparisons.

(e) *References.* For additional background information on this test guideline the following references shall be consulted:

(1) Adams, J., Buelke-Sam, J., Kimmel, C.A., Nelson, C.J., Reiter, L.W., Sobotka, T.J., Tilson, H.A., and Nelson, B.K. "Collaborative Behavioral Teratology Study: Protocol design and testing procedure." *Neurobehavioral Toxicology and Teratology* 7:579–586 (1985).

(2) Buelke-Sam, J., Kimmel, C.A., Adams, J., Nelson, C.J., Vorhees, C.V., Wright, D.C., St. Omer, V., Korol, B., Butcher, R.E., Geyer, M.A., Holson, J.F., Kutscher, C., and Wayne, M.J. "Collaborative Behavioral Teratology Study: Results." *Neurobehavioral Toxicology and Teratology* 7:591–624 (1985).

(3) Dixon, W.J. and Massey, E.J. *Introduction to Statistical Analysis.* 2nd Ed. New York: McGraw-Hill, (1957).

(4) Glowinski, J. and Iversen, L.L. "Regional studies of catecholamines in the rat brain—I." *Journal of Neurochemistry* 13:655–699 (1966).

(5) Jensen, D.R. "Some simultaneous multivariate procedures using Hotelling's T^2 Statistics." *Biometrics* 28:39–53 (1972).

(6) Kellogg, C., Tervo, D., Ison, J., Parisi, T., and Miller, R.K. "Prenatal exposure to diazepam alters behavioral development in rats." *Science* 207:205–207 (1980).

(7) Neter, J. and Wasserman: W. *Applied Linear Statistical Models.* Homewood, IL: Richard D. Irwin, Inc. (1974).

(8) Sokal, R.P. and Rohlf, E.J. *Biometry.* San Francisco: W.H. Freeman and Co. (1969).

(9) Tanimura, T. "Guidelines for developmental toxicity testing of chemicals in Japan." *Neurobehavioral Toxicology and Teratology* 7:647–652 (1985).

(10) Vorhees, C.V. "Comparison of the Collaborative Behavioral Teratology Study and the Cincinnati behavioral teratology test batteries." *Neurobehavioral Toxicology and Teratology* 7:625–633 (1985).

(11) Vorhees, C.V., Brunner, R.L., McDaniel, C.R., and Butcher, R.E. "The Relationship of gestational age to vitamin A induced postnatal dysfunction." *Teratolgoy* 17:271–276 (1978).

PART 799—[AMENDED]

2. In Part 799:

a. The authority citation continues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. New § 799.4440 is added to read as follows:

§ 799.4440 Triethylene glycol ethers.

(a) *Identification of test substances.* (1)Triethylene glycol monomethyl ether (CAS No. 112–35–6), triethylene glycol monomethyl ether (CAS No. 112–50–5), and triethylene glycol monobutyl ether (CAS No. 143–22–6) shall be tested in accordance with this section.

(2) Compounds of at least 90 percent purity shall be used as the test substances.

(b) *Persons required to submit study plans, conduct tests, and submit data.* (1) All persons who manufacture or process one or more of the listed chemicals, other than as an impurity, from the effective date of this section (44 days after the publication date of the final rule in the **Federal Register**) to the end of the reimbursement period shall submit letters of intent to conduct testing on the chemical or chemicals they manufacture or exemption applications, submit study plans, conduct tests in accordance with Part 792 of this chapter, and submit data as specified in this section, Subpart A of this Part, and Part 790 of this chapter.

(2) [Reserved].

(c) *Health effects testing*—(1) *Neurotoxicity*—(i) *Required testing.* Neurotoxicity tests shall be conducted in accordance with §§ 798.6050, 798.6200 and 798.6400 of this chapter with each of the chemicals listed in paragraph (a)(1) of this section. The tests shall be performed in the rat by dermal administration as specified under § 798.3300(b)(6)(ii) of this chapter for a period of 90 days.

(ii) *Modifications.* The following modification to these three sections shall apply: These three tests may be combined, using 10 animals per sex per dose level.

(iii) *Reporting requirements.* (A) The neurotoxicity tests shall be completed and final results submitted to the Agency within 15 months of the effective date of the final rule.

(B) Progress reports shall be submitted to the Agency at 6-month intervals, beginning 6 months after the effective date of the final rule.

(2) *Developmental toxicity*—(i) *Required testing.* Developmental toxicity tests shall be performed on rats and rabbits by dermal application as specified under § 798.3300(b)(6)(ii) of this chapter with each of the chemicals listed in paragraph (a)(1) of this section in accordance with § 798.4900 of this chapter.

(ii) *Reporting requirements.* (A) The developmental toxicity tests shall be completed and the results submitted to the Agency within 12 months of the final test rule.

(B) Progress reports shall be submitted to the Agency at 6-month intervals, beginning 6 months after the effective date of the final rule.

(3) *Developmental neurotoxicity*—(i) *Required testing.* Developmental neurotoxicity tests shall be performed in rats by dermal application as specified under § 798.3300(b)(6)(ii) of this chapter, with each of the chemicals listed in paragraph (a)(1) of this section, in accordance with § 795.250 of this chapter, following completion of the developmental toxicity study.

(ii) *Reporting requirements.* (A) The developmental neurotoxicity study shall be completed and the final results submitted to the Agency within 24 months of the effective date of the final test rule.

(B) Progress reports shall be submitted to the Agency at 6-month intervals, beginning 6 months after the receipt by the Agency of the final report of the developmental toxicity test.

(4) *Mutagenicity*—(i) *Required testing.* (A) An Ames test in *Salmonella* shall be done in accordance with § 798.5265 of this chapter for each of the chemicals listed in paragraph (a)(1) of this section.

(B) A gene mutation test in mammalian cells shall be done for each chemical listed in paragraph (a)(1) of this section as specified in § 798.5300 of this chapter if the results from the Ames test specified in paragraph (c)(4)(i)(A) of this section for that chemical are negative.

(C) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be performed for each chemical listed in paragraph (a)(1) of this section using the guidelines in § 798.5275 of this chapter if the results of either the Ames test specified in paragraph (c)(4)(i)(A) of this section or the mammalian cells in culture gene mutation assay as specified in paragraph (c)(4)(i)(B) of this section are non-negative for that chemical.

(D) An *in vitro* cytogenetics test shall be conducted in accordance with § 798.5375 of this chapter for each chemical listed in paragraph (a)(1) of this section.

(E) An *in vivo* cytogenetics test shall be done for each chemical listed in paragraph (a)(1) of this section by dermal absorption as specified under § 798.3300(b)(6)(ii) of this chapter, in accordance with § 798.5385 of this chapter if the *in vitro* test as specified in paragraph (c)(4)(i)(D) of this section for that chemical is negative.

(F) A dominant-lethal assay for each chemical listed in paragraph (a)(1) of this section shall be conducted by dermal application as specified under § 798.3300(b)(6)(ii) of this chapter, in accordance with § 798.5450 of this chapter if a non-negative result occurs in either the *in vitro* or *in vivo* cytogenetics test as specified in paragraphs (c)(4)(i)(D) and (E) of this section for that chemical.

(ii) *Reporting requirements.* (A) Mutagenicity tests shall be completed and final results submitted as follows: *Salmonella*, 5 months; mammalian cells in culture, 12 months; *Drosophila* sex-linked recessive lethal, 24 months; *in vitro* cytogenetics, 4 months; *in vivo* cytogenetics, 12 months; and dominant-lethal assay, 24 months.

(B) Progress reports are required at 6-month intervals, beginning 6 months after the effective date of the final rule.

(5) *Subchronic toxicity*—(i) *Required testing.* (A) A subchronic toxicity test shall be performed on the rabbit for each chemical listed in paragraph (a)(1) of this section by dermal application in accordance with § 798.2250 of this chapter.

(B) *Modifications.* The following modifications shall be incorporated in § 798.2250 of this chapter for testing each of the triethylene glycol ethers listed in paragraph (a)(1) of this section.

(*1*) *Observations.* The requirement under § 798.2250(e)(9)(iv) of this chapter is modified so that cage-side observations shall include daily examination for hematuria.

(*2*) *Hematology.* The requirement under § 798.2250(e)(10)(i)(A) of this chapter is modified so that hematology determinations shall be carried out 24 to 48 hours following initiation of dosing in addition to the other times specified. At all hematologic determinations additional measurements shall include a platelet count and mean corpuscular volume.

(*3*) *Clinical biochemistry.* The requirement under § 798.2250(e)(10)(i)(B) of this chapter is modified so that clinical biochemistry determinations shall be carried out 24 to 48 hours following initiation of dosing in addition to the other times specified.

(*4*) *Urinalysis.* The requirement under § 798.2250(e)(10)(ii)(B) of this chapter is modified so that urinalyses shall be done at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. The animals shall be kept in metabolism cages, and the urine shall be examined microscopically for the presence of erythrocytes and renal tubular cells, in addition to measurement of urine volume, specific gravity, glucose, protein/albumin and blood.

(*5*) *Liver-function tests.* The requirement under § 798.2250(e)(10)(ii) of this chapter is modified to add required testing for liver function using five rabbits per sex per dose with sulfobromophthalein (BSP) and a like number using indocyanine green (ICG). The same animals shall be tested at three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period.

(*6*) *Organ weights.* The requirement under § 798.2250(e)(11)(ii) of this chapter is modified so that the prostate gland, the epididymes, the seminal vesicles, and pituitary gland weights shall be determined wet, as soon as possible after dissection.

(*7*) *Gross pathology.* The requirement under § 798.2250(e)(11)(iii) of this chapter is modified so that the following additional organs shall be preserved in a suitable medium for future histopathologic examination: the vas deferenes, the oviducts and the vagina.

(*8*) *Histopathology.* The requirement under § 798.2250(e)(12)(i) of this chapter is modified so that the accessory genital organs (epididymes, prostate, seminal vesicles) and the vagina shall be examined histopathologically. In addition, preparations of testicular and associated reproductive organ samples for histology shall follow the recommendations of Lamb and Chapin (1985) under paragraph (d)(1) of this section, or an equivalent procedure, with particular attention directed toward achieving optimal quality in the fixation and embedding, and including an evaluation of the spermatogenic pattern. Spermatid counts shall be performed as described by Johnson *et al.* (1980) and Blazak *et al.* (1985) under paragraph (d) (2) and (3) of this section or an equivalent procedure. Epididymal sperm count and sperm morphology shall also be done.

(ii) *Reporting requirements.* (A) The subchronic test shall be completed and the final results submitted to the Agency within 15 months of the effective date of the final test rule.

(B) Progress reports shall be submitted at 6-month intervals, beginning 6 months after the rule is made final.

(d) *References.* For additional background information the following references should be consulted:

(1) Lamb, J.C. and Chapin, R.E. "Experimental models of male reproductive toxicology," *Endocrine Toxicology.* Eds. J.A. Thomas, K.S. Korach, J.A. McLachlan. New York, NY: Raven Press, pp. 85–115 (1985).

(2) Johnson, L., Petty, C.S., and Neaves, W.B. "A comparative study of daily sperm production and testicular composition in humans and rats," *Biology of Reproduction, 22:1233–1243. (1980).*

(3) Blazak, W.F., Ernest, T.L., and Stewart, B.E. "Potential indicators of reproductive toxicity: Testicular sperm production and epididymal sperm number, transit time and motility in Fischer 344 rats," *Fundamental and Applied Toxicology,* 5:1097–1103. (1985).

(Information collection requirements have been approved by the Office of Management and Budget under control number 2070–0033)

[FR Doc. 86–10704 Filed 5–14–86; 8:45 am]
BILLING CODE 6560–31–M